REMARKS

Claims 1, 3-5, 7, 9-13 and 19-21 are all the claims pending in the present application. Claims 1, 3-5, 7, 9-13 and 19-21 have been rejected. Claims 2, 6, 8, 14-18, 22-25 have been canceled. Claims 1, 7 and 19 are amended herewith.

Objections

The Examiner objected to the title of the invention as not being descriptive of the invention. A new title is submitted herewith. Applicants respectfully submit that the objection to the title on this basis should be withdrawn.

Claim Rejections under 35 U.S.C. §112, first paragraph

Claims 1, 3-5, 7, 9-13 and 19-21 have been rejected under 35 U.S.C. §112, first paragraph, as failing to comply with the written description requirements for the following reasons as set forth on pages 2-4 of the Office Action:

- the terms "nucleic acids" "sequence" "hybridize" and "coding portions" do not describe the structural features of the claimed nucleic acids;
- the structural and functional features of the claimed nucleic acids are not described;
- a nucleic acid that hybridizes to another may only have a small region in common and may encode a protein with a very different function;
- the specification does not describe non-AiiA proteins;
- not all nucleic acids that hybridize to any nucleic acid encoding SEQ ID NO:2 would encode an AiiA protein;

- the specification does not describe any signal peptide coding regions;
- "membrane attachment region" does not describe sequences of membrane attachment regions; and
- without deposit of bacterial isolate 240B1, "bacterial isolate 240B1" are simply words and the structural and functional features of the isolates are not described.

In response to the rejection, Applicants submit they have satisfied the written description requirement and have reasonably conveyed to one or ordinary skill in the art that Applicants had possession of the claimed invention. In an effort to advance prosecution and clarify the language of the claims, claims 1and 7 have been amended to include the limitation "encoding a bacterial autoinducer inactivation protein." Support for this amendment can be found at page 9, lines 29-31 of the specification. Such a limitation limits group member 1c) to an AiiA nucleic acid or protein sequence.

Regarding the Examiner's assertion that signal peptide coding regions are not described, one of skill in the art also understands that signal peptide coding regions are well known in the art. The specification discloses at page 9 that the nucleic acid optionally may further comprise a signal peptide coding region. Furthermore, examples of signal peptide coding regions of plants include tobacco and plant root exudates and are disclosed at page 10 of the specification. It is fundamental biology that signal peptides are about 20 amino acids and mark polypeptides of proteins destined for secretion. It is a peptide present on proteins that are destined either to be secreted or to be membrane components. It is usually at the N terminus and normally absent from the mature protein. *See Biology*: *Sixth Edition:* 320 (2002) attached herewith. At page 10 of the

specification, lines 25-31 describe pathogenic bacteria cells as confined to the intercellular area of plant tissue. Since it is desirable to target the aiiA protein into the intercellular spaces, a secretion signal peptide may be fused to the aiiA protein in order to accomplish this. The specification also discloses four references at lines 30-31 that describe such a process. Membrane attachment coding regions are also described in the specification. At pages 10-11 of the specification, lines 31-32 describe a plant membrane attachment motif as something that can be incorporated into the peptide sequence of AiiA for anchoring the AiiA enzyme in the outer surface of plant cell membranes.

With respect to "donor organisms," claim 19 has also been amended to further clarify the language of the claim. Claim 19, as amended, recites the limitation "bacterial isolates from plant and soil samples." Support for this amendment can be found at page 15, line 6 of the specification. Therefore, the donor organisms required by the method of claims 19-21 are fully described in the specification.

Accordingly, it is submitted that the specification provides an adequate written description of the claimed invention for claims 1 and 7 as amended and dependent claims 3, 5, 9, 11 and 19-21. Withdrawal of this rejection is requested.

Claims 1, 3-5, 7, 9-13 and 19-21 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for nucleic acids encoding SEQ ID NO:2, does not reasonably provide (according to the Examiner) enablement for nucleic acids that hybridize to SEQ ID NO: 1 or that hybridize to any nucleic acid that encodes SEQ ID NO:2, vectors comprising them, cells transformed with the vector and a method of using the nucleic acids to

increase disease resistance in a plant. The rejection has been repeated for the reasons of record as set forth in the Office Action mailed November 5, 2004.

Applicants submit that to satisfy the enablement requirement, the specification must teach those of skill in the art how to make and use the entire scope of the ***claimed*** invention without undue experimentation (emphasis added). *Genentech, Inc. v. Novo Nordisk, A/S*, 42 U.S.P.Q.2d 101, 1004 (Fed. Cir. 1997), *cert. denied*, 522 U.S. 963 (1997). The specification need not disclose what is well-known to those skilled in the art and preferably omits that which is well-known and already available to the public. M.P.E.P. 2164.05(a); *Spectra-Physics, Inc. V. Coherent, Inc.*, 3 U.S.P.Q.2d 1737, 1743 (Fed. Cir. 1987), *cert. denied*, 484 U.S. 954 (1987). Applicants are not required to provided detailed information concerning matters which are known in the prior art and well within the ordinary skill of a practitioner. *See* M.P.E.P. 2164.05(a). Even if the specification requires the skilled person in the art to engage in a "reasonable" amount of route experimentation, the specification complies with the enablement requirement so long as such experiment is not "undue." *In re Wands*, 8 U.S.P.Q.2d 1400, 1404 (Fed. Cir. 1988). A patent application is presumptively enabled when filed. *E.g., In re Marzocchi,* 169 U.S.P.Q. 367, 370 (C.C.P.A. 1971). "A specification that teaches how to make and use the invention in terms which correspond in scope to the claims must be taken as complying with the first paragraph of 35 U.S.C. 112 unless there is reason to doubt the objective truth of the statements relied upon therein for enabling support." *Id.* The "examiner has the initial burden to establish a reasonable basis to question the enablement provided for the **claimed** invention." M.P.E.P. 2164.04; *In re Wright*, 27 U.S.P.Q.2d 1510, 1513 (Fed. Cir. 1993)

(emphasis added). In *Wright*, the Court made clear that the PTO has the burden of providing a reasonable explanation of why the specification does not enable. If the examiner is able to provide such evidence or reasoning, she has established a *prima facie* case of nonenablement. *See* M.P.E.P. 2164.04.

One of skill in the art knows that nucleic acid hybridization is a process where the nucleic acid base pairs with a complimentary sequence of another nucleic acid molecule. The temperature and salt concentrations at which hybridization is performed has a direct effect upon the results that are obtained. Specifically, you can set the conditions up so that your hybridizations only occur between the probe and a filter bound nucleic acid that is highly homologous to that probe. Furthermore, claims 1 and 7, as amended include the limitation "encoding a bacterial autoinducer inactivation protein." Therefore, one of skill in the art would know that the nucleic acid encoding a bacterial autoinducer inactivation protein of part c) would base pair to either the nucleic acid encoding a bacterial autoinducer inactivation protein having the sequence of the coding portion of SEQ ID NO: 1 or the nucleic acid encoding a bacterial autoinducer inactivation protein encoding the amino acid sequence of SEQ ID NO: 2. Both sequences for SEQ ID NO: 1 and SEQ ID NO: 2 are disclosed in Figures 4(a) and 4(b).

The hybridization conditions in part c) of claim 1 are designed to hybridize aiiA derivatives with more than 80% (Eff Tm of aiiA) homology at the DNA sequence level. One of skill in the art could easily perform such a calculation based on a standard formula provided at the website,

http://www.ndsu.nodak.edu/instruct/mcclean/plsc731/dna/dna6.htm.

The website page is attached herewith.

Standard Formula

Eff T_m=81.5+16.6(log M [Na+])+0.41(%G+C)

As aiiA=37% GC, 1XSSC[Na+]M=0.165, then

Eff T_m of aiiA= 81.5+16.6(log 0.165)+0.41 (37%) =81.5-13+15.17=83.5.

Since it has been well established that a 1% mismatch of two DNA molecules lowers the T_m 1.4°C, when temperature of hybridization is at 55° C, the minimum homology of the DNA target to the probe (aiiA) can be calculated using 83.5% from the calculation above:

% Homology=100-[(83.5-55)/1.4]=100-20.36=79.6%

As a result, the conditions stated in part c) of claim 1 are stringent to ensure hybridization of aiiA derivatives with high homologies (>79%).

Applicants submit that the Examiner has not provided acceptable evidence to doubt the objective enablement of the specification and to support her contention that the specification is not enabling. Accordingly, it is submitted that the specification provides enablement for the claimed invention. Withdrawal of this rejection is requested.

The Examiner argues that the specification allegedly does not describe the transformation of any plant with a nucleic acid that hybridizes to SEQ ID NO:1 or that hybridizes to any nucleic acid that encodes SEQ ID NO:2; therefore, it would be undue trial and error to screen through the myriad of nucleic acids encompassed by the claims and plants transformed therewith, to identify those with increased disease resistance, if such plants are even obtainable. However, in *In re Borkowski*, 164 U.S.P.Q. 642, 645 (C.C.P.A. 1970), the court reasoned that a specification need

not even contain a single working example to be considered enabling. Even so, the present invention relates to a method for increasing disease resistance in a plant or animal, by introducing into the cell of the plant or animal a nucleic acid sequence which encodes a bacterial autoinducer inactivation protein in a manner which allows the cell to express the nucleic acid sequence. *See* page 5, lines 18-24. It would not be undue trial and error to screen through the nucleic acids encompassed by the claims because the nucleic acid that hybridizes to SEQ ID NO:1 or that hybridizes to a nucleic acid encoding SEQ ID NO:2 is a nucleic acid that encodes a bacterial autoinducer inactivation protein. The specification also identifies plants with increased disease resistance. Diseases of various plants including potato, cabbages, tomato, chili, carrot, celery, onion, and lettuce are known in the art. *See* pages 19-20. Any molecular biologist of ordinary skill can easily identify the nucleic acids encompassed by the claims and utilize well-known hybridization techniques to transform a diseased plant.

Accordingly, it is submitted that the specification provides enablement for the claimed invention. Withdrawal of this rejection is requested.

The specification, according to the Examiner does not teach from which organisms the nucleic acid of claim 1, part c) can be isolated, or which organisms can be used as donor organisms in the method of claims 19-21, and also argued that bacterial isolate 240B1 cannot be used to isolate the nucleic acid of SEQ ID NO:1 because it is not deposited or publicly available. The Examiner also reiterates the argument that the specification does not describe the isolation of a nucleic acid that hybridizes to SEQ ID NO:1 or that hybridizes to any nucleic acid that encodes SEQ ID NO:2 from a publicly available donor organism, and undue trial and error

experimentation would be required to screen all the donor organisms encompassed by the claims to identify those that have a nucleic acid that hybridizes to SEQ ID NO:1 or that hybridizes to any nucleic acid that encodes SEQ ID NO:2.

It would not be undue trial and error experimentation to isolate a nucleic acid encoding a bacterial autoinducer inactivation protein that hybridizes to SEQ ID NO:1 or that hybridizes to a nucleic acid encoding a bacterial autoinducer inactivation protein that encodes SEQ ID NO:2 from a publicly available donor organism, because the sequence for *Bacillus* sp. 240B1 is publicly available. Furthermore, the specification does teach from which organism the nucleic acid of claim 1, part c) can be isolated, or which organisms can be used as donor organisms in the method of claims 19-21. Page 15 of the specification discloses bacterial isolates from plant and soil samples. It would be reasonable using standard molecular biology techniques and a computer to screen for soil isolates for acyl homoserine lactone degrading activity.

Accordingly, it is submitted that the specification provides enablement for the claimed invention. Withdrawal of this rejection is requested.

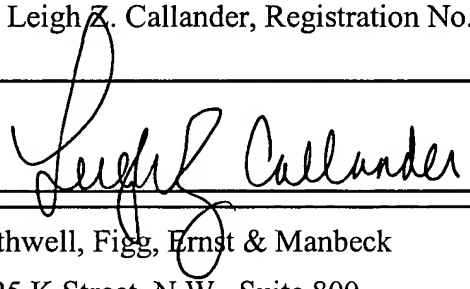
Claim Rejections under 35 U.S.C. §112, second paragraph

Claims 1, 3-5 and 19-21 are rejected under 35 U.S.C. §112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter that Applicant regards as the invention. The rejection is repeated for reasons of record as set forth in the Office Action mailed November 5, 2004.

The Examiner argues that any nucleic acid has at least 6 potential reading frames, and thus at least 6 coding portions. Applicants respectfully disagree. Figure 4A teaches the nucleotide sequence of the aiiA gene [SEQ ID NO: 1]. The specification discloses the coding portion as starting at base 1. Bases 1-3 in Figure 4 stand for the DNA codon ATG which one of skill in the art knows is the mRNA start codon AUG. The termination site is labeled by a thick underline *See* page 7, lines 27-30. Claim 1 part (a) distinctly claims the coding portion of SEQ ID NO: 1. Those skilled in the art would understand the scope of the claim when the claim is read in light of the specification. *North American Vaccine Inc. v. American Cyanamide Co.*, 28 USPQ2d 1333, 1339 (Fed. Cir. 1993)(citing *Orthokinetics, Inc. v. Safety Travel Chairs,* 1 USPQ.2d 1081, 1088 (Fed. Cir. 1986)) *cert. denied*, 511 US. 1069 (1994).

For the above reasons, Applicants respectfully submit that the claims distinctly claim the subject matter of the present invention and request that the rejection under 35 U.S.C. §112, second paragraph be withdrawn.

In view of the above amendments and remarks, it is submitted that the claims are in condition for allowance. The Examiner is invited to telephone the undersigned to expedite allowance of this application.

RESPECTFULLY SUBMITTED,			
Name and Reg. Number	Leigh Z. Callander, Registration No. 55,035		
Signature		DATE	September 26, 2005

Address	Rothwell, Figg, Ernst & Manbeck 1425 K Street, N.W., Suite 800				
City	Washington	*State*	D.C.	*Zip Code*	20005
Country	U.S.A.	*Telephone*	202-783-6040	*Fax*	202-783-6031

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Nucleic Acid Hybridizations

The hybridization of a radioactive probe to filter bound DNA or RNA is one of the most informative experiments that is performed in molecular genetics. Two basic types of hybridizations are possible.

Southern hybridization - hybridization of a probe to filter bound DNA; the DNA is typically transferred to the filter from a gel

Northern hybridization - hybridization of a probe to filter bound RNA; the RNA is typically transferred to the filter from a gel

Probes are the primary tool used to identify complementary sequences of interest. Generally, the probe is a clone developed by inserting DNA into a vector. Most often these are plasmid clones.

Probe - a single-stranded nucleic acid that has been radiolabelled and is used to identify a complimentary nucleic acid sequence that is membrane bound

The hyrbridization process involves two different steps. First the nucleic acid must be immobilized on a filter. This is generally called a "Southern Transfer" procedure. The second step is the actual hybridization of the probe to the filter bound nucleic acid.

The following steps describe the Southern transfer procedure.

1. Digest DNA with the restriction enzyme of choice.

2. Load the digestion onto a agarose gel and apply an electrical current. DNA is negatively charged so it migrates toward the "+" pole. The distance a specific fragment migrates is inversely proportional to the fragment size.

3. Stain the gel with EtBr, a fluorescent dye which intercalates into the DNA molecule. The DNA can be visualized with a UV light source to assess the completeness of the digestion.

4. Denature the double-stranded fragments by soaking the gel in alkali (>0.4 M NaOH).

5. Transfer the DNA to a filter membrane (nylon or nitrocellulose) by capillary action. Typically a

Southern transferst setup contains (from bottom to top):

- buffer
- sponge
- filter paper
- the gel containing the nucleic acid
- a nylon or nitrocellulose membrane
- more filter paper
- paper towels to catch the buffer that passed through all of the above

Southern hybridizations with plant DNA is not a trivial matter. The primary requirement for a successful experiment is that the DNA to be probed is digested to completion. We have already discussed the choice of enzymes in this regard. Even when using compatible enzymes (not GC or GXC sensitive) monitoring the completeness of the reaction is essential for consistent results.

Once you are satisfied that you completely digested the DNA and are confident that it was successfully transferred to the filter membrane, the next step is perform the actual hybridization. The following steps describe the procedure.

Steps in Southern Hybridization Procedure

1. Prepare a probe by nick translation or random, oligo-primed labelling.

2. Add the probe to a filter (nylon or nitrocellulose) to which single-stranded nucleic acids are bound. (The filter is protected with a prehybridization solution which contains molecules which fill in the spots on the filter where the nucleic acid has not bound.

3. Hybridize the single-stranded probe to the filter-bound nucleic acid for 24 hr. The probe will bind to complementary sequences.

4. Wash the filter to remove non-specifically bound probe.

5. Expose the filter and determine:
   - **a. Did binding occur?**
   - **b. If so, what is the size of hybridizing fragment?**

Hybridization Stringency

The temperature and salt concentrations at which we perform a hybridization has a direct effect upon the results that are obtained. Specifically, you can set the conditions

up so that your hybridizations only occur between the probe and a filter bound nucleic acid that is highly homologous to that probe. You can also adjust the conditions the hybridzation is to a nucleic acid that has a lower degree of homology to the probe.

Your hybridization results are directly related to the number of degrees below the Tm of DNA at which the experiment is performed. For a aqueous solution of DNA (no salt) the formula for Tm is:

T_m = 69.3°C + 0.41(% G + C)°C

From this formula you can see that the GC content has a direct effect on Tm. The following examples, demonstrate the point.

T_m = 69.3°C + 0.41(45)°C = 87.5°C (for wheat germ)

T_m = 69.3°C + 0.41(40)°C = 85.7°C

T_m = 69.3°C + 0.41(60)°C = 93.9°C

Hybridizations though are always performed with salt. This requires another formula which that takes the salt concentration into account. Under salt-containing hybridization conditions, the effective Tm is what controls the degeree of homology between the probe and the filter bound DNA is required for successful hybridization. The formula for the Effective Tm (Eff T_m).

Eff T_m = 81.5 + 16.6(log M [Na+]) + 0.41(%G+C) - 0.72(% formamide)

The salt solution that is most often used in hybridization experiments is SSC (standard sodium citrate). Different concentrations of this solution are used at different steps in the hybridization procedure. The following table gives the Na+ concentration for different strengths of SSC. Remember that this value is essential to derive the Eff T_m.

Na+ ion concentration of different strengthsof SSC

SSC Content	[Na+] M
20X	3.3000
10X	1.6500
5X	0.8250
2X	0.3300
1X	0.1650
0.1X	0.0165

Another relevant relationship is a that ***1% mismatch of two DNAs lowers the T_m 1.4°C.*** So in a hybridization with wheat germ that is performed at T_m - 20°C (=67.5°C), the two DNAs must be 85.7% homologous for the hybridization to occur. 100% - (20oC/1.4°C) = 85.7% homology

Let's now look at an actual experiment, the hybridization of a probe with filter bound wheat DNA in 5X SSC at 65oC. The first step is to derive the Eff T_m.

Eff T_m = 81.5 + 16.6(log 0.825) + 18.5 = 98.6°C

These types of hybridization experiments are typically performed at T_m - 20°C. A typical temperature of hybridization is 65°C. (If formamide is used the hybridization is normally performed at 42°C). With these conditions, 83.1% homology between the probe and filter bound DNA is required for hybridization. The following calculation is how this number was derived.

100 - [(98.6-65.0)/1.4] = 100 - (23.6/1.4) = 83.1%.

The next step in a hybridization experiment is to wash the filter. This is normally done in two steps. First a non-stringent wash is performed to remove the non-specifically bound DNA and the second wash is performed at a higher stringency that only permits highly homologous sequences to remain bound to the filter. Controlling the stringency is an important step in these experiments.

Stringency - a term used in hybridization experiments to denote the degree of homology between the probe and the filter bound nucleic acid; the higher the stringency, the higher percent homology between the probe and filter bound nucleic acid

Non-stringent wash: normally 2X SSC, 65°C

Eff T_m = 81.5 + 16.6[log(0.33)] + 0.41(45%)= 92.0°C

%Homology = 100 - [(92-65)/1.4] = 80.7%

Stringent wash: normally 0.1X SSC, 65°C

Eff T_m = 81.5 + 16.6[log(0.0165)] + 0.41(45%) = 70.4°C

%Homology = 100 -[(70.4-65)/1.4] = 96.1%

This example shows that the final wash is the one of

concern when determining the relatedness of the probe and the filter bound nucleic acid.

An example: Bowman-Kirk Protease Inhibitor Final wash is performed at 0.2X SSC, 55oC; assume 45% GCcontent

Eff T_m = 81.5 + 16.6[log(0.033)] + 18.5 = 75.4^oC

%Homology = 100 - [(75.4-55.0)/1.4] = 85.4%

The point to this last example to emphasize that your percent homology is directly related to your most stringent condition in your hybridization experiment. This invariably is the final wash. Thus, you only need to make this calculation to determine the stringency of your experiment.

What You Can Learn from Southern Hybridizations

Southern hybridizations have many applications. The first application after cloning a gene is often to determine how many copies of the gene are in the species from which the gene was cloned. This experiment is performed by hybridizing a clone of the gene to total DNA that has been digested with several enzymes. The procedure is termed a ***genomic southern.***

One gene that has drawn intense interest because of its potential applied usage in plant biotechnology is chitinase. We have already discussed the isolation of a clone for this gene from bean. As you can imagine, the gene has also been cloned from other species. The first page of hybridization handout shows the southern hybridization pattern obtained from cucumber, rice and bean. These hybridizations show that these species contain ***different copy numbers*** for the gene.

A second application for southern hybridizations is the estimation of copy number of a specific gene. This experiment is performed by running several lanes with different copy numbers of the gene to which you are probing and comparing the hybridization intensities with a companion genomic southern experiment. This is called a ***reconstruction experiment.*** The example in on the second page of the handout is for phaseolin, the major storage protein of bean. In this example, 1, 2 and 5 genomic equivalents (copies per bean genome) are seen in lanes 7-9. The other lanes are various restriction digestions of total bean DNA. After hybridization, densitometric readings were taken and it was determined that bean contained 6.5 copies of the gene. This agreed with the data obtained from reassociation kinetic experiments.

Southern hybridization analysis can also be performed to

determine if a phenotypic mutation is due to a structural change in the gene controlling the trait of interest. If a gene undergoes an insertion or deletion the resulting hybridization pattern would be changed. Insertional mutagenesis would generate fragments of an increased size whereas deletions would reduce the size of the hybridizing band. Two tomato mutants, Neverripe (nr) and ripening inhibitor (ri) express polygalacturonase, an enzyme involved in fruit ripening, at
lower levels than normal or wild type tomato. The question posed here was whether the structure of the polygalacturonase (and other ripening specific genes) are structurally different than the wild type genotype. The thrid page of the handout. shows that the structure of these genes in the mutatnt is not different than the wild type. Therefore, some other molecular event is responsible for these differences in expression patterns of the different mutants.

some cases, a single polypeptide chain may be enzymatically cleaved into two or more pieces. For example, the protein insulin is first synthesized as a single polypeptide chain but becomes active only after an enzyme cuts out a central part of the chain, leaving a protein made up of two polypeptide chains connected by disulfide bridges (see FIGURE 5.22). In other cases, two or more polypeptides that are synthesized separately may join to become the subunits of a protein that has quaternary structure.

Signal peptides target some eukaryotic polypeptides to specific destinations in the cell

In electron micrographs of eukaryotic cells active in protein synthesis, two populations of ribosomes (and polyribosomes) are evident: free and bound (see FIGURE 7.10). Free ribosomes are suspended in the cytosol and mostly synthesize proteins that dissolve in the cytosol and function there. In contrast, bound ribosomes are attached to the cytosolic side of the endoplasmic reticulum (ER). They make proteins of the endomembrane system (the nuclear envelope, ER, Golgi apparatus, lysosomes, vacuoles, and plasma membrane) as well as proteins secreted from the cell. Insulin is an example of a secretory protein. The ribosomes themselves are identical and can switch their status from free to bound.

What determines whether a ribosome will be free in the cytosol or bound to rough ER at any particular time? The synthesis of all proteins begins in the cytosol, when a free ribosome starts to translate an mRNA molecule. There the process continues to completion—*unless* the growing polypeptide itself cues the ribosome to attach to the ER. The polypeptides of proteins destined for the endomembrane system or for secretion are marked by a **signal peptide,** which targets the protein to the ER (FIGURE 17.21). The signal peptide, a sequence of about 20 amino acids at or near the leading (amino) end of the polypeptide, is recognized as it emerges from the ribosome by a protein-RNA complex called a **signal-recognition particle (SRP).** This particle functions as an adapter that brings the ribosome to a receptor protein built into the ER membrane. This receptor is part of a multiprotein complex. Polypeptide synthesis continues there, and the growing polypeptide snakes across the membrane into the cisternal space via a protein pore. The signal peptide is usually removed by an enzyme. The rest of the completed polypeptide, if it is to be a secretory protein, is released into

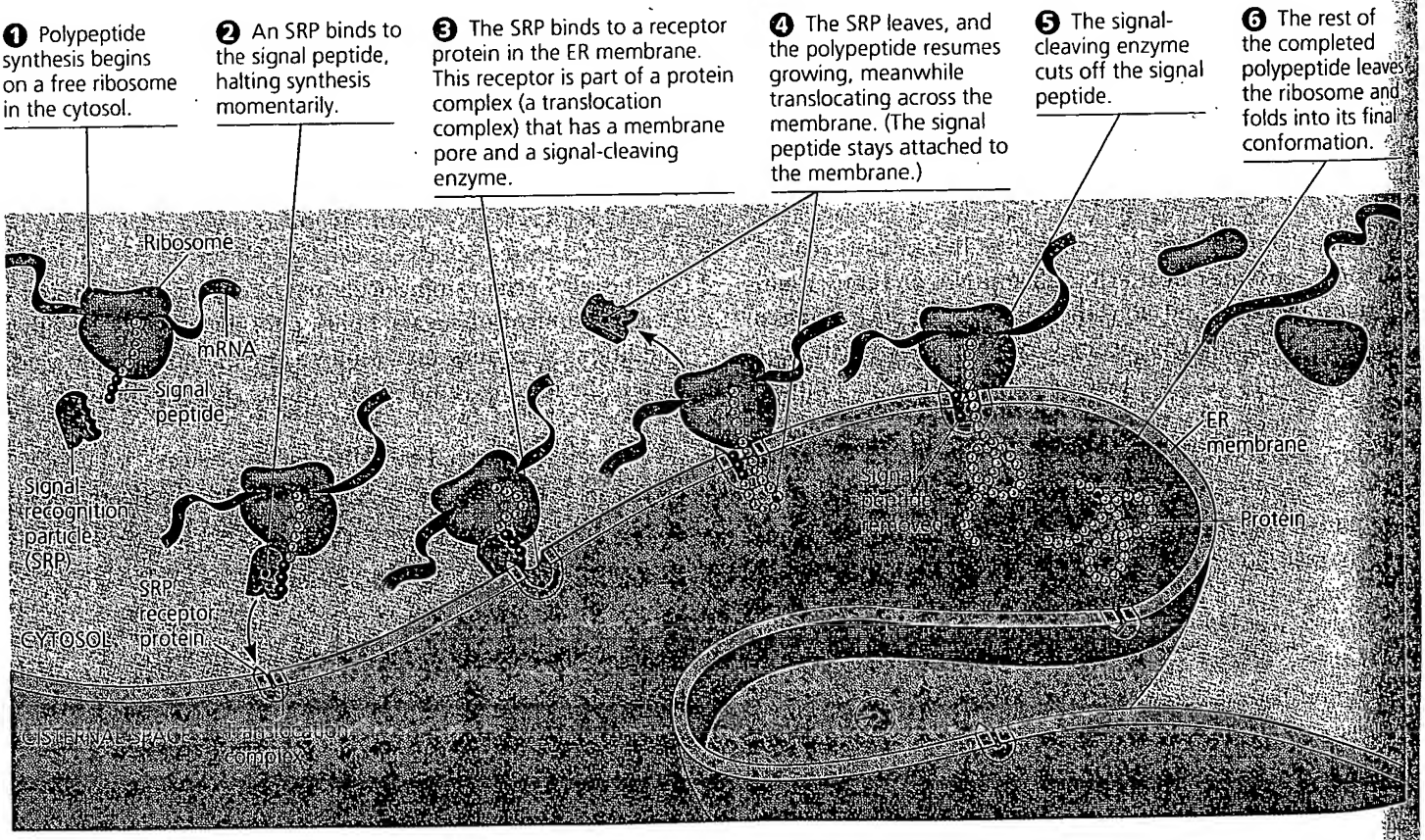

FIGURE 17.21 The signal mechanism for targeting proteins to the ER. A polypeptide destined for the endomembrane system or for secretion from the cell begins with a signal peptide, a series of amino acids that targets it for the ER. This figure shows the synthesis of a secretory protein and its simultaneous import into the ER. In the ER and then in the Golgi, the protein is further processed. Finally, a transport vesicle conveys it to the plasma membrane for release from the cell (see FIGURE 8.8).

solution within the cisternal space (as in FIGURE 17.21). Alternatively, if the polypeptide is to be a membrane protein, it remains partially embedded in the ER membrane.

Other kinds of signal peptides are used to target polypeptides to mitochondria, chloroplasts, the interior of the nucleus, and other organelles that are not part of the endomembrane system. The critical difference in these cases is that translation is completed in the cytosol before the polypeptide is imported into the organelle. The mechanisms of translocation also vary, but in all cases studied to date, the "postal" codes that address proteins to cellular locations are signal peptides of some sort.

RNA plays multiple roles in the cell: *a review*

As we have seen, the cellular machinery of protein synthesis (and ER targeting) is dominated by RNA of various kinds. In addition to mRNA, these include tRNA, rRNA, and, in eukaryotes, snRNA and SRP RNA (TABLE 17.1). The diverse functions of these molecules range from structural to informational to catalytic. The ability of RNA to perform so many different functions is based on two related characteristics of this kind of molecule: RNA can hydrogen-bond to other nucleic acid molecules (DNA or RNA), and it can assume a specific three-dimensional shape by forming hydrogen bonds between bases in different parts of its polynucleotide chain (you saw an example of this intramolecular bonding in tRNA, FIGURE 17.13). DNA may be the genetic material of all living cells today, but RNA is much more versatile. You will learn in Chapter 18 that many viruses even use RNA rather than DNA as their genetic material.

Table 17.1 Types of RNA in a Eukaryotic Cell

Type of RNA	Functions
Messenger RNA (mRNA)	Carries information specifying amino acid sequences of proteins from DNA to ribosomes
Transfer RNA (tRNA)	Serves as adapter molecule in protein synthesis; translates mRNA codons into amino acids
Ribosomal RNA (rRNA)	Plays catalytic (ribozyme) roles and structural roles in ribosomes
Primary transcript	Serves as a precursor to mRNA, rRNA, or tRNA and may be processed by splicing or cleavage. In eukaryotes, pre-mRNA commonly contains introns, noncoding segments that are spliced out as the primary transcript is processed. Some intron RNA acts as a ribozyme, catalyzing its own splicing.
Small nuclear RNA (snRNA)	Plays structural and catalytic roles in spliceosomes, the complexes of protein and RNA that splice pre-mRNA in the eukaryotic nucleus
SRP RNA	Is a component of the signal-recognition particle (SRP), the protein-RNA complex that recognizes the signal peptides of polypeptides targeted to the ER

Comparing protein synthesis in prokaryotes and eukaryotes: *a review*

Although bacteria and eukaryotes carry out transcription and translation in very similar ways, we have noted certain differences in cellular machinery and in details of the processes. Prokaryotic and eukaryotic RNA polymerases are different, and those of eukaryotes depend on transcription factors. Transcription is terminated differently in the two kinds of cells. Also, prokaryotic and eukaryotic ribosomes are slightly different. The most important differences, however, arise from the eukaryotic cell's compartmental organization. Like a one-room workshop, a prokaryotic cell ensures a streamlined operation. In the absence of a nucleus, it can simultaneously transcribe and translate the same gene (FIGURE 17.22), and the newly made protein can quickly diffuse to its site of function.

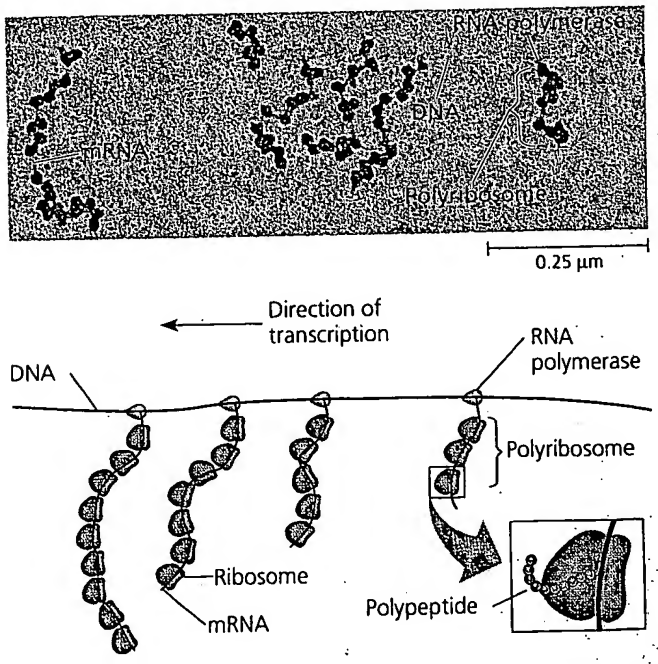

FIGURE 17.22 Coupled transcription and translation in bacteria. In prokaryotic cells, the translation of mRNA can begin as soon as the leading (5′) end of the mRNA molecule peels away from the DNA template. The micrograph shows a strand of *E. coli* DNA being transcribed by RNA polymerase molecules. Attached to each RNA polymerase molecule is a growing strand of mRNA, which is already being translated by ribosomes. The newly synthesized polypeptides are not visible in the micrograph (TEM).